Molecular Epidemiology of Hereditary Epidermolysis Bullosa in a Middle Eastern Population

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Epidermolysis bullosa (EB) encompasses a large group of inherited blistering skin disorders caused by mutations in at least 10 genes. Numerous studies, mainly performed in European and US families with EB, have revealed a number of characteristic epidemiological and genetic features, which form the basis for current diagnostic and counseling strategies. However, little is currently known about the molecular epidemiology of EB in Middle East populations. In the present study, we assessed 55 EB families for pathogenic sequence alterations in the 10 genes known to be associated with EB. Our results show unique EB subtype distribution and patterns of inheritance in our cohort. We also failed to detect recurrent mutations frequently encountered in Europe and the US, and did not consistently observe genotype–phenotype correlations formerly established in Western populations. Thus, the molecular epidemiology of EB in the Middle East is significantly different from that previously delineated in Europe and the US. Our data raise the possibility that similar differences may also be found in other genetically heterogeneous groups of disorders, and indicate the need for population-specific diagnostic and management approaches.

Journal of Investigative Dermatology (2006) **126,** 777–781. doi:10.1038/sj.jid.5700163; published online 26 January 2006

INTRODUCTION

Epidermolysis bullosa (EB) is defined as a large, heterogeneous group of inherited mechanobullous disorders (Uitto and Richard, 2005) resulting from mutations in genes coding for various components of the cutaneous basement membrane zone, a complex structure that anchors the epidermis to the underlying dermis (Ghohestani *et al.*, 2001).

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Abbreviations: DEB, dystrophic EB; EB, epidermolysis bullosa; EBS, EB simplex; JEB, junctional EB

Received 15 September 2005; revised 4 November 2005; accepted 23 November 2005; published online 26 January 2006

EB is one of the most common inherited skin disorders with a population prevalence of 19 in 1 million in the US, 32 in 1 million in Northern Ireland, and 49 in 1 million in Scotland (McKenna *et al.*, 1992; Horn *et al.*, 1997; Fine *et al.*, 1999). Prevalence rates in Middle East populations are unknown; however, the high frequency of consanguineous unions characteristic of these populations (Zlotogora, 1997) suggests that recessive types of EB may be more common in this region. EB is associated with a high mortality rate and a number of local and systemic complications, such as infections, failure to thrive, anemia, esophageal and urethral strictures, amyloidosis, and cancer (Uitto and Richard, 2005). Therefore, EB generates a significant burden on medical health systems, a problem of particular importance in underprivileged areas of the world.

More than 30 different clinical EB variants have been described (Fine *et al.*, 2000), most of which can be assigned to three major histopathological subtypes, as defined by the microscopic location of the blisters: EB simplex (EBS; MIM 131800, 131760, 131900), junctional EB (JEB; MIM 226700, 226650), and dystrophic EB (DEB; MIM 131750, 226600), characterized by blister formation within the epidermal basal cell layer, the lamina lucida, and the upper dermis, respectively. The majority of the families with EBS show autosomal dominant inheritance pattern; all forms of JEB are transmitted in an autosomal recessive fashion, while inheritance in DEB can be either autosomal recessive or autosomal dominant (Uitto and Richard, 2005).

To date, mutations in 10 distinct genes have been found to cause EB (Uitto and Richard, 2005). Each of the proteins encoded by these 10 genes plays a critical role in the function of the basement membrane, and abnormal function of any of these results in a defined EB subtype with a fairly predictable associated phenotype (Marinkovich, 1999; Uitto and Pulkkinen, 2001). Mutations in the genes encoding keratins 5 and 14 (KRT5 and KRT14) have been shown to underlie EBS; mutations in the genes encoding the three subunit polypeptides of laminin 5 (LAMA3, LAMB3, and LAMC2) have been shown to cause JEB; and mutations in the gene coding for type VII collagen (COL7A1) have been shown to result in DEB (Uitto and Richard, 2005). Five additional genes have been reported to harbor mutations in less frequent variants of EB: mutations in PLEC1, coding for plectin, cause EB with late-onset muscular dystrophy (MIM 226670), the Ogna variant of EBS (MIM 131950), EB with pyloric atresia (MIM 226730), and a recently described lethal form of JEB (Charlesworth *et al.*, 2003; Pfendner *et al.*, 2005); mutations in ITGA6 and ITGB4, which code for the two subunits of integrin $\alpha 6\beta 4$, may be responsible for EB with pyloric atresia, but also for EBS or JEB without gastrointestinal manifestations (Inoue *et al.*, 2000; Jonkman *et al.*, 2002); mutations in COL17A1, coding for type XVII collagen, were found to cause generalized atrophic benign EB, a nonlethal variant of JEB (Bauer and Lanschuetzer, 2003; Uitto and Richard, 2005), as well as a mild form of EBS (Huber *et al.*, 2002).

In the past, the majority of genetic studies of EB have focused on European and US populations, and little is currently known about the clinical and molecular features of this group of diseases in other parts of the world, including the Middle East. Several preliminary reports (Nakano *et al.*, 2002b; Ciubotaru *et al.*, 2003; Indelman *et al.*, 2005) have suggested that the demographic features specific to the Middle East may underlie a number of unique epidemiological and biological attributes characteristic of EB patients in this region. In the present study, we report the cumulative data obtained for 55 EB families originating from Israel and Palestine.

RESULTS AND DISCUSSION

Distribution of EB subtypes and clinical features

A total of 55 families diagnosed with EB on the basis of typical clinical and histopathological features underwent a formal genetic analysis (Tables S1–S3). EBS, JEB, and DEB represented 40, 27, and 33% of the cohort, respectively (Figure 1a). As compared with data previously collected in various countries (ie Japan, Norway, Northern Ireland, and United States) (McKenna *et al.*, 1992; Fine *et al.*, 1999) (Figure 1b), the percentage of dominant EBS cases in the present series was significantly low. This may have resulted from the high proportion of recessive EB subtypes in our cohort, which in turn may be due to the high inbreeding coefficient characteristic of populations of the Middle East (Zlotogora, 1997). This assumption is supported by the observation that only 48% of the Israeli EB patients were of Jewish background, despite the fact that the Jewish population, characterized by a low rate of consanguineous marriages, accounts for more than 80% of the Israeli population (http://www.mfa.gov.il/MFA/MFAArchive/2000_2009/2004/).

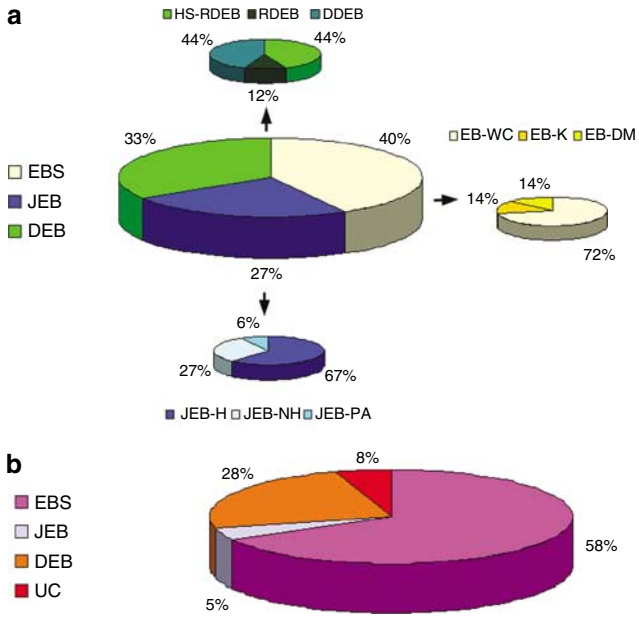

Figure 1. Distribution of the various EB subtypes. The data collected in the present study are shown in (**a**). The data reported by the National EB Registry (Fine *et al.*, 1999) are shown for comparison in (**b**). EB-WC, EBS Weber–Cockayne type characterized by involvement of hands and soles; EB-K, EBS Koebner type, characterized by generalized involvement; EB-DM, EBS Dowling–Meara type, characterized by generalized herpetiform skin blistering accompanied by mucosal involvement; JEB-H, JEB Herlitz type characterized by perinatal lethality; JEB-NH, JEB non-Herlitz type, characterized by survival through early childhood; JEB-PA, JEB associated with pyloric atresia; HS-RDEB, recessive DEB Hallopeau–Siemens type characterized by extensive skin and mucosal blistering and scarring; RDEB, recessive DEB; DDEB, dominant DEB; UC, unclassified.

According to a study in Northern Ireland (McKenna *et al.*, 1992) and the report of the National EB Registry (Fine *et al.*, 1999), JEB represents approximately 2–5% of all EB cases in Western populations. However, this EB subtype accounted for 27% of the cases in our series, once again pointing to the higher prevalence of recessive disorders in Middle East populations (Alwan and Modell, 2003), albeit JEB appears to be relatively rare in the Israeli Jewish population (Table S2). Of note, in a previous survey of 49,902 patients in a general clinic in Saudia, no JEB cases were reported (Abahussein *et al.*, 1993). However, it is not clear how the diagnosis of the various EB subtypes was approached and if it was skewed towards non-lethal EB subtypes, since the study was conducted in an outpatient setting, thus underestimating the prevalence of severe/lethal JEB cases.

For the DEB subtype, the proportion of DEB cases relative to the total number of families was comparable to that reported in the US population (Fine *et al.*, 1999) (Table S3). However, a significantly higher percentage of the cases

displayed a recessive mode of inheritance as compared with that recorded by the National EB Registry (Fine *et al.*, 1999).

Molecular features

Mutation analysis was concordant with the histopathological diagnosis in all cases. A total of 50 different mutations were identified in the 55 families of our cohort, 17 of which have not been previously reported.

EB simplex (Table S1). Altogether, 22 EBS cases were assessed. In all, 14 cases (64%) were due to mutations in KRT14, while only a minority of the cases was caused by sequence alterations in KRT5 (36%).

Autosomal recessive EBS, the least prevalent type of EBS in Western countries with only a few sporadic cases reported (reviewed in Indelman *et al.*, 2005), represented in our cohort approximately one-third of EBS cases. Mutations W305X and R134C were carried by patients of Arab Moslem origin, while Q396X most probably originated from Transylvania and was found in families of Jewish Ashkenazi origin only (Ciubotaru *et al.*, 2003; Indelman *et al.*, 2005).

We identified two patients with EBS harboring a heterozygous mutation, R388H, which was previously reported in a recessive case of EBS (Ciubotaru *et al.*, 2003). No other mutations in KRT5 or KRT14 were detected in these two affected individuals and R388H was not found in more than 200 population-matched control individuals (400 chromosomes). Moreover, the R388 residue is highly conserved among keratin molecules (ConSeq score = 9; range 1–9; http://conseq.bioinfo.tau.ac.il). Despite this strong evidence for R388H being a disease-causing mutation, R388H was also detected in the healthy father of two affected children carrying R388H and a maternal nonsense mutation in KRT14 (Ciubotaru *et al.*, 2003), suggesting lack of penetrance for R388H or underlying paternal mosaicism in this specific kindred.

In Western populations, mutations affecting codon 125 of KRT14 have been shown to cause more than 40% of all EBS cases (Sybert and Stephens, 1994; Fine *et al.*, 1999); however, only two such cases (9%) were identified in our series. This result emphasizes again the profound differences in the molecular epidemiology of EB between Middle Eastern and Western populations.

Junctional EB (Table S2). In all, 15 families with JEB were assessed, and a total of 15 mutations were identified. Previous data, mainly collected in US and European populations, had revealed the following facts: most mutations causing JEB are found in the *LAMB3* gene; two hot spot mutations, R635X and R42X, account for 50% of the mutations carried by JEB patients; nonsense/frameshift mutations, in contrast to missense mutations, are almost always associated with a poor prognosis; and mutations in COL17A1 are associated with a benign disease course (Nakano *et al.*, 2000, 2002b; Bauer and Lanschuetzer, 2003).

Mutation analysis performed in our cohort failed to confirm these previous findings. For example, mutations were almost evenly distributed between the four JEB genes: *LAMA3* (27%), *LAMB3* (33%), *LAMC2* (13%), and *COL17A1* (20%), with a single case associated with PA being caused by mutations in ITGB4. In addition, the hot spot mutations R635X and R42X, causing JEB without pyloric atresia, were found in 0 of 30 and 1 of 30 mutant alleles, respectively. Finally, there was no definitive correlation between the nature of the mutation-carrying gene or the type of the causative mutation and the course of the disease, thus confirming our previous preliminary data (Nakano *et al.*, 2002b): nonsense and frameshift mutations resulted in a lethal phenotype and missense mutations caused a nonlethal phenotype in eight out of 11 cases due to mutations in laminin 5-encoding genes (Table S2). In addition, we report here the first case of lethal JEB caused by a homozygous mutation in COL17A1, 4144del4. Since the affected child carrying this mutation received intensive treatment in a modern tertiary hospital, the severe course of his disease cannot be attributed to poor medical care. Of note, the parents of this patient reported that a second child in this family, who was not examined, also died shortly after birth due to extensive skin blistering.

Dystrophic EB (Table S3). Of the 18 DEB cases caused by deleterious mutations in COL7A1 (Table S3), 11 (61%) displayed a recessive mode of inheritance, while the remaining were inherited in an autosomal dominant manner. In one recessive case, despite sequencing of the entire coding region of the gene, only one mutation could be identified, suggesting the existence of an additional causative mutation in noncoding regions of the gene. Half of the mutations identified in our patients were novel, suggesting that COL7A1 mutations are often unique to individual families or ethnic groups (Salas-Alanis *et al.*, 2000; Murata *et al.*, 2004). However, the hot spot mutation G2043R (Christiano *et al.*, 1995) was identified in two Jewish families. In contrast to our results for EBS and JEB in this cohort, genotype–phenotype correlation analysis of the DEB cases did not reveal unusual findings. Recessive inheritance was often predictive of poor prognosis, as reported previously (Gardella *et al.*, 2002).

Implication for EB patient care in the Middle East

The results of this study carry important implications for the care of EB patients in the Middle East.

First, genetic counseling of families at risk for EB should rely upon a combined clinical, histological, and molecular analysis. For example, the inheritance pattern of EBS cannot be determined based on clinico-pathological grounds alone (Table S1), and requires molecular confirmation due to the relatively high prevalence of autosomal recessive keratin mutations. Conversely, the presence of specific genetic mutations in an EB family does not always allow predicting the course and outcome of the disease, calling for caution in interpreting these data for the purpose of genetic counseling. Indeed, in this and a previous study (Nakano *et al.*, 2002b), we identified JEB cases due to nonsense mutations, which did not result in a lethal phenotype as generally assumed. On the other hand, we report here a case of lethal JEB caused by a mutation in COL17A1, despite the fact that all previously

reported mutations in this gene were associated with a rather benign course (Bauer and Lanschuetzer, 2003).

Second, diagnostic strategies based upon molecular epidemiological features determined in populations of Western origin may not be applicable to Middle Eastern populations, possibly due to marked demographic differences, in particular consanguineous marriages. The low prevalence of known hot spot mutations (eg R125H/C in KRT14, R635X in LAMB3) as well as the lack of preponderance of mutations in given genes (eg LAMB3 in JEB) (Tables S1 and S2) indicate the need for specific diagnostic algorithms adapted to the molecular features of EB in Middle Eastern populations. Indeed, our data suggest that mutation analysis should be prioritized according to ethnic (eg Q196X in KRT14 in patients of Jewish Austro-Hungarian origin) or geographic (eg Q1083X in LAMB3 in patients of Arab Moslem origin living in Northern Israel) origins of EB patients.

Third, the fact that genotype–phenotype correlations firmly established in Western populations are not consistently observed in a Middle Eastern population suggests the existence of inherited, epigenetic, or environmental modifier traits, which are responsible for modulating the clinical expression of pathogenic mutations in EB, as has previously been shown in some individual cases in the past (McGrath *et al.*, 1999; Bodemer *et al.*, 2003). The identification of these factors in Middle East populations may be instrumental in developing a comprehensive strategy for the counseling of families at risk for EB in this geographic region.

MATERIALS AND METHODS

Patients and biological materials

Families were recruited in Israel over a period of 5 years by actively searching all available registries at our institutions as well as by publicizing our efforts during professional meetings. All participants or their legal guardian provided written and informed consent according to a protocol previously approved by the local Helsinki Committee and by the National Committee for Genetic Human Research of the Israeli Ministry of Health in accordance with the Declaration of Helsinki Principles. Blood samples were drawn from all family members and DNA was extracted according to standard procedures. EB diagnosis and subtyping were determined based on light microscopy and electron microscopy examination of skin biopsies as described previously (Bergman, 1999; Petronius *et al.*, 2003).

Mutation analysis

Genomic DNA was PCR-amplified using primer pairs encompassing all exons and exon–intron boundaries of the 10 genes known to be associated with EB as described previously (McGrath *et al.*, 1995a; Kon *et al.*, 1997; Takizawa *et al.*, 1997; Rouan *et al.*, 2000; Nakano *et al.*, 2002a; Ciubotaru *et al.*, 2003). For the *KRT5* and *KRT14* genes, amplicons were immediately gel-purified (QIAquick gel extraction kit, Qiagen, Valencia, CA) and subjected to bidirectional DNA sequencing using the BigDye terminator system on an ABI Prism 3100 sequencer (PE Applied Biosystems, Foster City, CA). DNA sequence analysis was performed using the Sequencher platform. For mutational screening in other genes (*LAMA3*, *LAMB3*, LAMC2, *COL17A1*, *ITGB4*, *ITGA6*, *PLEC*, and *COL7A1*), we used conformation-sensitive gel electrophoresis or dHPLC (Transgenomics, Omaha, NE) to screen PCR products for heteroduplex formation prior to direct sequencing (Pfendner *et al.*, 2003). All novel mutations were excluded from at least 100 control individuals using either direct sequencing or PCR-restriction fragment length polymorphism.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We acknowledge the patients and their families for participation in the present study. We are grateful to V. Friedman for DNA sequencing services. This study was supported in part by a grant provided by the Bureau for Economic Growth, Agriculture, and Trade, Office of Economic Growth and Agricultural Development, the US Agency for International Development, under the terms of Award No. TA-MOU-01-M21-023 (E.S., M.K., J.U., G.R., R.B.).

SUPPLEMENTARY MATERIAL

Table S1. Mutation analysis in EBS families.

Table S2. Mutation analysis in JEB families.

Table S3. Mutation analysis in DEB families.

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